

## FINE STRUCTURE OF A SCYPHOZOAN PLANULA, *CASSIOPEIA XAMACHANA*

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### ABSTRACT

Pre-metamorphic planulae of the scyphozoan *Cassiopeia xamachana* contain four cell types. The ectoderm consists of supportive cells and differentiating nematoblasts and nematocytes, while the endoderm consists of supportive cells and interstitial cells. Neural elements and glandular cells are absent in these planulae. Morphological similarities and differences that exist among hydrozoan, scyphozoan, and anthozoan planulae are discussed.

### INTRODUCTION

Most cnidarians have a planula stage at some time in their life cycle. Planulae are cylindrical and are composed of an ectoderm and an endoderm separated by a thin mesoglea. In recent years several ultrastructural studies have described the morphology of hydrozoan and anthozoan planulae (Lyons, 1973a, b; Vandermeulen, 1974; Chia and Crawford, 1977; Martin and Thomas, 1977, 1980; Chia and Koss, 1979). The ultrastructural morphology of scyphozoan planulae has been largely ignored. Otto (1978) examined the morphological and ultrastructural changes which took place during settlement of scyphozoan planulae of *Haliclystus salpinx*. The planulae of this Stauromedusae are atypical in that they lack cilia, do not swim, and usually contain a constant number of endodermal cells. Since there has been no comprehensive fine-structural study to date describing a more typical scyphozoan planula, we examined the planulae of *Cassiopeia xamachana*. It is hoped that such a study might reveal possible morphological similarities and differences among hydrozoan, scyphozoan, and anthozoan planulae.

### MATERIALS AND METHODS

Adult *Cassiopeia* were collected in December, 1980 at La Paguera, Puerto Rico. Gonads and gastric filaments were removed from the adults, placed in finger bowls of filtered sea water, and macerated with a pipette. Young planulae were soon observed swimming in these containers. Four days after collection of planulae, swimming planulae were fixed for 2½ hours in 2.5% glutaraldehyde in 0.2 M phosphate buffer (Dunlap, 1966; Cloney and Florey, 1968). They were post-fixed for 2 hours in 2% osmium tetroxide, pH 7.2, in 1.25% sodium bicarbonate (Wood and Luft, 1965). Specimens for transmission electron microscopy were dehydrated in an ethanol series, infiltrated with propylene oxide, and embedded in Epon (Luft, 1961). Blocks were sectioned on a Porter Blum MT-2B ultramicrotome, placed on 150-mesh copper grids, and stained in 5% uranyl acetate in methanol followed by lead hydroxide. Grids were examined with a Phillips EM 201 transmission electron

microscope. Planulae fixed for scanning electron microscopy were dehydrated through a graded series of amyl acetates, critical point dried, mounted on stubs and shadowed with carbon followed by gold. The specimens were examined with a Cambridge Stereoscan 150 SEM.

For histochemical studies and the detection of glandular cells, thick plastic serial sections, 1–3  $\mu\text{m}$  thick, were mounted on glass slides. The Epon was removed according to the method of Lane and Europa (1965) and the sections were stained by the periodic acid-Schiff (PAS) procedure (Lillie, 1954).

## RESULTS

The pre-metamorphic planula of *Cassiopeia* ranges from 120  $\mu\text{m}$  to 220  $\mu\text{m}$  in length and from 85  $\mu\text{m}$  to 100  $\mu\text{m}$  in width in its mid-region. It is uniformly ciliated and swims with the enlarged anterior end forward. Just prior to metamorphosis, an indentation is found at the anterior end (Fig. 1). The majority of planulae observed settle on the bottoms of glass dishes and undergo metamorphosis within 4–5 days after collection. In some cases, planulae undergo metamorphosis without prior attachment to glass.

Fine-structural examination of pre-metamorphic planulae reveals only 4 cell types: 2 in the ectoderm and 2 in the endoderm. The ectoderm consists of supportive cells and differentiating nematoblasts and nematocytes. Supportive cells are columnar in shape and extend from the free surface of the planula to the mesoglea (Fig. 2). Each supportive cell bears microvilli and a single cilium at its apical surface (Figs. 2 and 3). The cilium is of the 9 + 2 microtubular arrangement and extends from the apical surface without a concavity. It consists of a basal plate located above a basal body and an accessory basal body (Fig. 4). The basal body gives rise to a striated ciliary rootlet with a periodic banding pattern of about 300 Å. The rootlet extends deep into the cytoplasm of the cell and terminates just above the nucleus. Attached to the accessory basal body is a plaque-like structure that parallels the ciliary rootlet (Fig. 4). Microfilaments of a terminal web are found directly beneath the apical surfaces of the cells (Fig. 5) and terminate at the lateral cell boundaries on either side. Septate desmosomes are present between these supportive cells in their apical regions (Fig. 6). Numerous electron-dense, membrane-bounded granules fill the apical regions of the cells (Fig. 2). Vacuoles are also present. The nucleus of each cell is centrally located and contains a nucleolus and condensed chromatin. A few Golgi complexes are located in close association with the nucleus. Mitochondria, polysomes, and endoplasmic reticulum are scattered throughout the cytoplasm.

Basally, foot processes of the supportive cells insert on the mesoglea (Fig. 7). PAS-positive granules and glycogen particles are abundant in the basal regions of these cells. Specialized junctional complexes resembling desmosomes and hemidesmosomes are located between the foot processes of adjacent supportive cells and between the foot processes and the mesoglea (Fig. 8). Microfilaments are seen radiating out from dense regions located along the inner borders of the junctional membranes (Fig. 9). The two membranes are separated by a space of 150–200 Å.

Fully differentiated nematocytes are abundant at the ectodermal surfaces of planulae (Fig. 10). They are especially numerous in the anterior indentation region. The cells are embedded within the supportive cells and do not extend to the mesoglea. The nematocyst is large and occupies the upper two-thirds of the cell. A modified cilium gives rise to the cnidocil which is located to the side of the nematocyst. The capsule of the nematocyst consists of an outer electron-dense layer

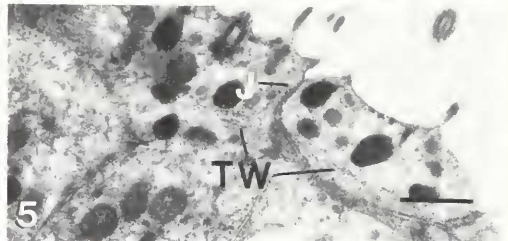
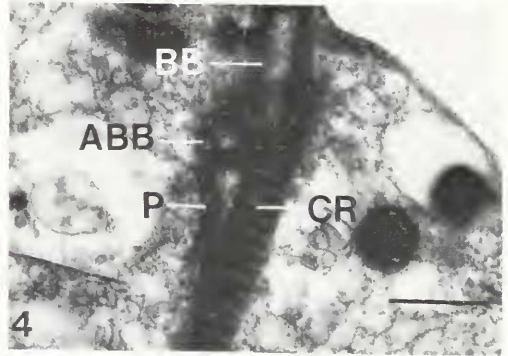
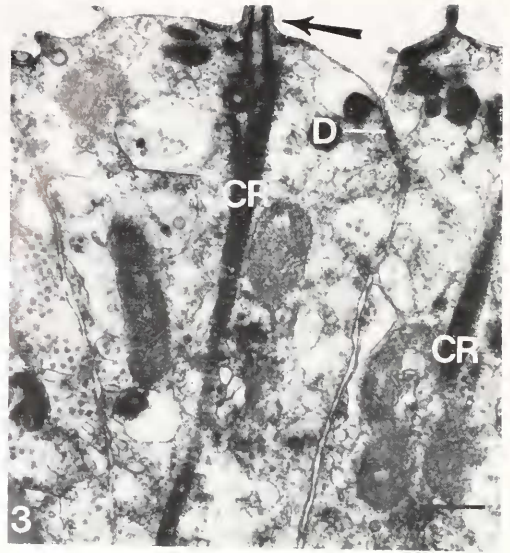
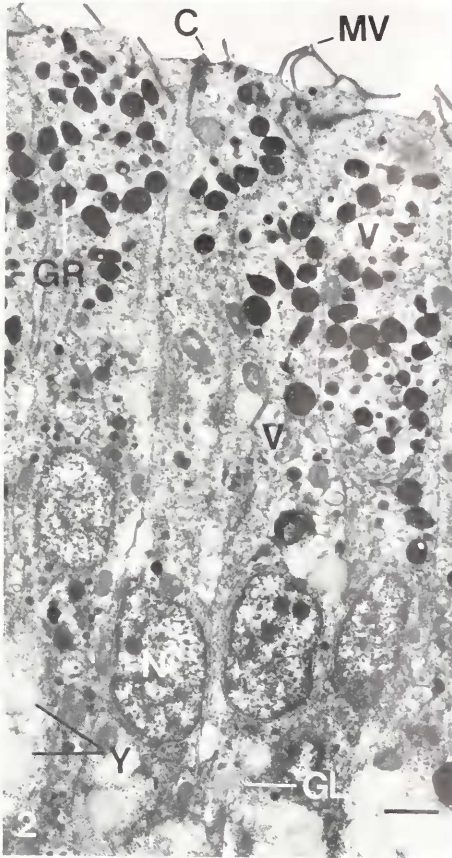


FIGURE 1. Scanning electron micrograph of a planula of *Cassiopeia*. The planula has a distinct anterior end and posterior end and is uniformly ciliated. Just prior to attachment and metamorphosis, an indentation is found in the anterior end (arrow). A = anterior; P = posterior. Bar = 20  $\mu$ m.

FIGURE 2. Transverse section of the apical regions of ectodermal supportive cells. These supportive cells possess a single cilium, numerous microvilli, and electron-dense granules. The nucleus of the cell is centrally located and contains a prominent nucleolus. PAS-positive granules and glycogen particles are located more basally in these cells. C = cilium; GL = glycogen particles; GR = granules; MV = microvilli; N = nucleus of supportive cell; V = vacuoles; Y = PAS-positive granules. Bar = 2  $\mu$ m.

FIGURE 3. Cilium of ectodermal supportive cell. Each cilium projects directly from the apical membrane of the cell without a concavity (arrow). The ciliary rootlet extends deep into the cytoplasm of the cell. CR = ciliary rootlet; D = desmosome. Bar = 1  $\mu$ m.

FIGURE 4. Basal body (BB), accessory basal body (ABB), ciliary rootlet (CR), and plaque-like structure (P) of a supportive cell. Bar = 1  $\mu$ m.

FIGURE 5. Terminal web beneath the apical membrane of a supportive cell. The microfilaments of the terminal web insert at the cell junctions. J = junction between cells; TW = terminal web. Bar = 2  $\mu$ m.

TABLE I

*Comparison of planulae.*

| Class  | Anthozoa                         |                            |                               | Hydrozoa                   |   | Scyphozoa                         |                            |                             |
|--|----------------------------------|----------------------------|-------------------------------|----------------------------|---|-----------------------------------|----------------------------|-----------------------------|
|  | <i>Anthopleura elegantissima</i> | <i>Ptilosarcus gurneyi</i> | <i>Pocillopora damicornis</i> | <i>Balanophyllia regia</i> | <i>Pennaria tiarella</i>                | <i>Mitrocomella polydiademata</i> | <i>Halichystus salpinx</i> | <i>Cassiopeia xamachana</i> |
| References                                   | Chia and Koss (1979)             | Chia and Crawford (1977)   | Vandermeulen (1974)           | Lyons (1973)               | Martin and Thomas (1977); Martin (1980) | Unpublished observations          | Otto (1978)                | Unpublished observations    |
| Age (post-fertilization in days)             | 21                               | 18                         | 6 (?)                         | (?)                        | 3-6                                     | 3-5                               | 5                          | 3-6                         |
| Size ( $\mu\text{m}$ ) length $\times$ width | 200 $\times$ 150                 | 1,000 $\times$ 300         | 1,000 $\times$ 500            | (?)                        | 650 $\times$ 80                         | 200 $\times$ 70                   | 100 $\times$ 20            | 120 $\times$ 85             |
| Types of cells in ectoderm                   | 9                                | 7                          | 9                             | 10                         | 7                                       | 7                                 | 3                          | 2                           |
| Types of cells in endoderm                   | 6                                | 2                          | 1 (?)                         | 1 (?)                      | 2                                       | 2                                 | 1 (16 cells)               | 2                           |
| Uniformly ciliated                           | Yes                              | Yes                        | Yes                           | Yes                        | Yes                                     | Yes                               | No                         | Yes                         |

(flagellated)

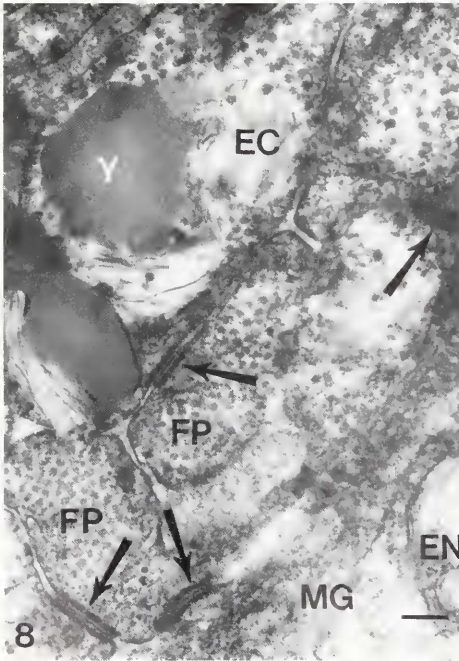
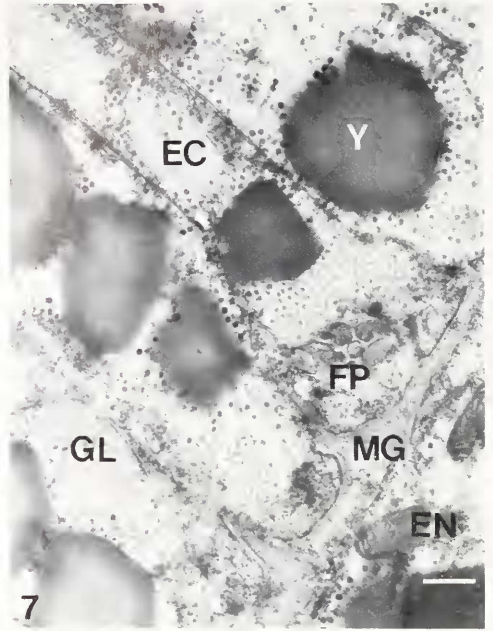
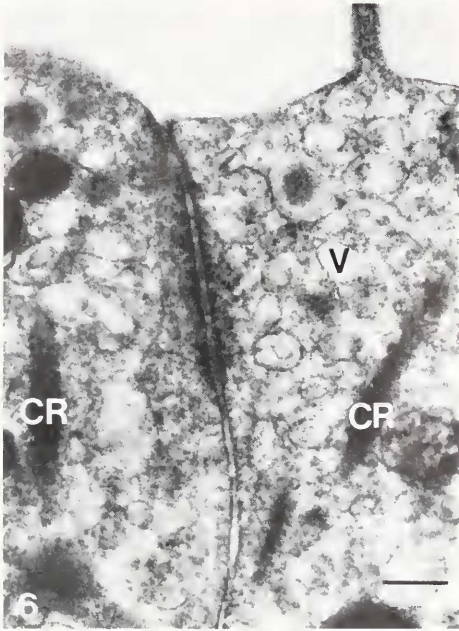


FIGURE 6. Septate desmosome between 2 supportive cells. CR = ciliary rootlet; V = vacuole. Bar = 0.5  $\mu$ m.

FIGURE 7. Foot processes of the ectodermal supportive cells. These processes insert on the mesoglea and contain numerous PAS-positive granules and glycogen particles. EC = ectoderm; EN = endoderm; FP = foot process of supportive cell; GL = glycogen particles; MG = mesoglea; Y = PAS-positive granules. Bar = 1  $\mu$ m.

and an inner electron-lucent layer. The thread of the nematocyst bears arms and spines and is coiled around a heavily barbed shaft. The nucleus of the cell is small and basally located. A well-developed Golgi apparatus is also present in the basal region of the cell. Endoplasmic reticulum is scarce.

Developing nematoblasts are located at the base of the epidermis among the foot processes of the supportive cells (Fig. 11). They do not make contact with the free surface of the ectoderm. The cytoplasm of these developing nematoblasts contains endoplasmic reticulum, a Golgi complex, and a developing nematocyst.

The endoderm is composed of a single layer of columnar-shaped cells very similar in structure to the supportive cells of the ectoderm (Fig. 12). These cells insert on the mesoglea *via* their basal ends. PAS-positive granules, vacuoles, glycogen particles, and electron-dense granules are abundant in these basal regions. Microfilaments are not detected in these cells. Apically the cells bear a single cilium surrounded by a collar of microvilli which projects into a forming gastrovascular cavity (Figs. 12 and 13). The nucleus is centrally located and often contains a nucleolus. Mitochondria, endoplasmic reticulum, and polysomes are found throughout the cytoplasm.

Clusters of interstitial cells are scattered among these supportive cells of the endoderm. The nucleus of each interstitial cell is round and contains a prominent nucleolus. Numerous free ribosomes are present in a homogeneous cytoplasm. Other organelles are sparse or poorly developed.

A thin mesoglea separates the ectoderm from the endoderm. The mesoglea consists of a meshwork of fibers which are oriented in all directions. These fibers are embedded within a PAS-positive, amorphous ground substance.

Examination of thick plastic serial sections and comparable thin sections repeatedly demonstrate the absence of both nerve cells and glandular cells in planulae of *Cassiopeia*. The negative PAS staining reaction also verifies the absence of glandular cells.

#### DISCUSSION

Results from this study and that of Otto (1978) indicate that planulae of scyphozoans are smaller in size and are morphologically simple when compared to planulae of hydrozoans and anthozoans (Table 1). Planulae of *Cassiopeia* and *Haliclystus* are composed of only 4 cell types, whereas the hydrozoan planulae thus far examined contain 9 cell types, and the anthozoan planulae possess anywhere from 9 to 15 cell types. In *Cassiopeia* the planular ectoderm consists of 1 type of supportive cell and 1 type of nematocyte, while the endoderm contains interstitial cells and 1 kind of supportive cell. In *Haliclystus* 3 types of cells are present in the ectoderm (1 form of supportive cell, 1 form of nematocyte, and interstitial cells), and only 1 type of supportive cell comprises the endoderm. Otto (1978) reported microfilaments at the base of the supportive cells in both ectoderm and endoderm of planulae of *Haliclystus*. In planulae of *Cassiopeia*, however, microfilaments were found only

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FIGURE 8. Specialized junctional complexes (arrows) between the foot processes of adjacent supportive cells and between the foot processes and the mesoglea. EC = ectoderm; EN = endoderm; FP = foot process of supportive cell; MG = mesoglea; Y = PAS-positive granules. Bar = 1  $\mu$ m.

FIGURE 9. Specialized junctional complex between 2 foot processes of the supportive cells. These junctions are very similar to desmosomes in that the unit membranes appear thickened due to the presence of a dense amorphous layer closely applied to their cytoplasmic surfaces. Microfilaments (arrows) radiate out from this amorphous substance. A slender intermediate dense line is seen in the middle of the intercellular space between the 2 halves of the junction. Bar = 0.5  $\mu$ m.

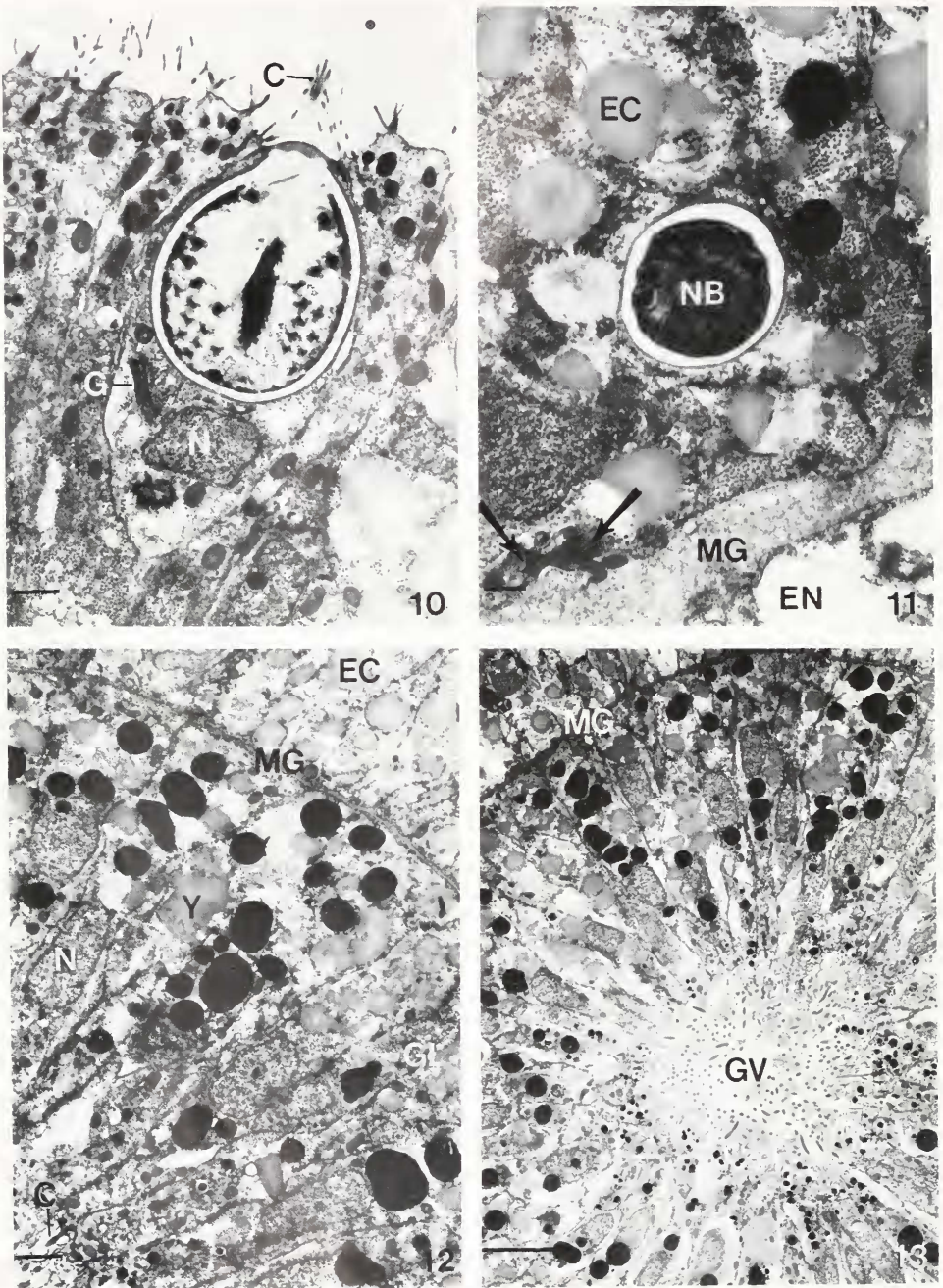


FIGURE 10. Nematocyte at the ectodermal surface of the planula. The nematocyte contains a large nematocyst and a basally located nucleus. A well-developed Golgi body is usually found in a supranuclear position. C = cilium; G = Golgi body; N = nucleus. Bar = 2  $\mu$ m.

FIGURE 11. Developing nematoblast located at the base of the ectoderm among the foot processes of the supportive cells. Specialized junctions between the foot processes of the supportive cells can be seen (arrows). EC = ectoderm; EN = endoderm; MG = mesoglea; NB = nematoblast. Bar = 1  $\mu$ m.

in the apical cytoplasm and the foot processes of the ectodermal supportive cells. Both the planulae of *Cassiopeia* and *Haliclystus* lack glandular cells and neural elements which generally are present in hydrozoan and anthozoan planulae (Lyons, 1973a, b; Vandermeulen, 1974; Chia and Crawford, 1977; Martin and Thomas, 1977, 1980; Chia and Koss, 1979).

Comparisons of the ultrastructural morphology of planulae from the 3 classes of cnidarians may add important insights into the phylogenetic classification of the cnidarians. Planulae of *Pennaria* (Martin and Thomas, 1977, 1980) and *Mitrocormella* (Martin *et al.*, unpublished observations) have 7 types of cells in the ectoderm and 2 kinds of cells in the endoderm. These hydrozoans are similar to the scyphozoan planulae in that in both classes the supportive cells of the ectoderm and the endoderm are arranged in a simple columnar epithelium with basal foot processes that insert on a thin mesoglea. Also, in both classes the planulae contain only 1 type of nematocyte. The 2 classes differ in that the hydrozoans contain neurosensory cells, ganglionic cells, and 2 types of glandular cells in the ectoderm. Anthozoan planulae, when compared to planulae of hydrozoans and scyphozoans, tend to show an increase in the types of glandular cells, the types of supportive cells, the types of nematocytes, and the complexity of the nervous system. The ectoderm of anthozoan planulae may be simple columnar, pseudostratified, or stratified depending upon the species examined (Vandermeulen, 1974; Chia and Crawford, 1977; Lyons, 1973a). Planulae of *Ptilosarcus* have 2 types of supportive cells and 3 types of glandular cells in the ectoderm (Chia and Crawford, 1977). In *Pocillopora* and *Balanophyllia* 3 types of nematocytes and 4 kinds of secretory cells are found in the ectoderm of the planulae (Vandermeulen, 1974; Lyons, 1973a, b). Planulae of *Anthopleura* possess 3 types of glandular cells in the ectoderm, and they exhibit the most complicated nervous system described to date for a planula larva (Chia and Koss, 1979). The nervous system consists of an apical organ, 1 type of endodermal receptor cell, 2 types of ectodermal receptor cells, inter-neurons, and a nerve plexus.

Werner (1973), in his analysis of the evolution of the cnidarian classes, proposed that the stem form of the recent cnidarians was a solitary, sessile, tetramerous polyp. He postulated that the Anthozoa were an early offspring from this common ancestor, and that the Scyphozoa, Hydrozoa, and Cubozoa arose from another evolutionary line. The acceptance of Werner's concept would result in the classification of the phylum Cnidaria into 2 subphyla: Anthozoa and Medusozoa. In the Anthozoa the polyp is the sexual adult and a medusa never develops, whereas, in the Medusozoa a medusa is the normal sexual adult and the polyp is regarded as a larval stage. The Medusozoa would consist of the extinct class Conulata and the recent classes Scyphozoa, Hydrozoa, and Cubozoa. Based on the comparative fine-structural morphology of the planulae examined to date, planulae of scyphozoans and hydrozoans appear to be more closely related to each other than are planulae of the scyphozoans and anthozoans or planulae of the hydrozoans and anthozoans (Koss, personal communication). The cells which comprise hydrozoan and scyphozoan planulae

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FIGURE 12. Transverse section of the supportive cells of the endoderm. Foot processes of these cells insert on the mesoglea. PAS-positive granules, vacuoles, glycogen particles, and electron-dense granules are found in the basal regions of the cells. The cells possess a single cilium surrounded by microvilli which projects into the gastrovascular cavity. The nucleus of the cell is centrally located. C = cilium; EC = ectoderm; GL = glycogen particles; MG = mesoglea; N = nucleus of supportive cell; Y = PAS-positive granules. Bar = 2  $\mu$ m.

FIGURE 13. Transverse section of the gastrovascular cavity of a planula. Numerous cilia and microvilli from the endodermal supportive cells project into the lumen of the cavity. GV = gastrovascular cavity; MG = mesoglea. Bar = 5  $\mu$ m.



are morphologically similar and are not as complex in their overall structural design as are the cells of anthozoan planulae. Some anthozoan planulae are provided with spirocysts which are absent in planulae of hydrozoans and scyphozoans. Furthermore, many anthozoan planulae possess an apical organ. Such a structure has not been reported in a hydrozoan or scyphozoan planula.

It is our judgment that in the future many more cnidarian biologists will turn their attention to the comparative cytology of planulae. It is expected that results from such investigations will contribute new ideas to both the developmental biology of the cnidarians and to the phylogenetic classification of the cnidarians.

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